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Soil remediation with a microbial community established on a carrier: Strong hints for microbial communication during 1,2,4-Trichlorobenzene degradation

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HIGHLIGHTS

- During 1,2,4-TCB mineralization signal molecules are produced.
- Microbial communities attached on a carrier establish a cell-to-cell communication.
- Efficient cell-to-cell communication results in high 1,2,4-TCB mineralization.
- Quantity and quality of signaling molecules depend on the type of inoculation.
- Cell-to-cell communication occurs via interspecies communication.

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ABSTRACT

The objective of the present study was to get more insight into the mechanisms that govern the high mineralization potential of a microbial community attached on a carrier material, as we found in an earlier study (Wang et al., 2010). A 1,2,4-Trichlorobenzene (1,2,4-TCB) degrading microbial community - attached (MCCP) and non-attached (MCLM) on clay particles – was inoculated into a simplified mineral medium system. Signaling molecules (AHLs), cell growth and 1,2,4-TCB mineralization were measured at different sampling points. The production of AHLs in the MCCP system increased continuously with increasing key degrader (*Bordetella* sp.) cell growth and a positive correlation was observed between the production of AHLs and 1,2,4-TCB mineralization. In the MCLM system, however, 1,2,4-TCB mineralization was lower than in the MCCP system; the AHLs production per *Bordetella* cell was higher than in MCCP and there was no correlation between AHLs and mineralization. Moreover, in the MCCP system less different AHLs were produced than in the MCLM system. These results indicate that a microbial community attached on a carrier material has an advantage over a non-attached community: it produces signaling molecules with much less energy and effort to achieve a well-directed cell-to-cell communication resulting in a high and effective mineralization.

1. Introduction

Numerous reports exist worldwide on the contamination of soils with organic chemicals and resulting risks for human health, and therefore many scientists were and are still discussing the pros and cons of various bio-augmentation approaches for decontaminating soils. Remarkable reviews on this wide topic have been presented (Gentry et al., 2004; Fantroussi and Agathos, 2005). When using microbial induced approaches for soil decontamination, various inoculation techniques are available: (i) isolated microbial strains are applied to soils; (ii) a combination of various and very efficient strains is used; (iii) specific microbial communities which were extracted from an adapted soil are inoculated; (iv) the microorganisms are introduced into soils as free cells or (v) as cells established on carrier materials.

One of the main problems of such bio-augmentation approaches is their sustainability. In laboratory experiments the survival rate of inoculated microbes whether inoculated as free cells or as cells established on a carrier into sterilized soil materials even

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at relatively low chemical concentrations is not critical because of the lacking competition between inoculated and native soil microbes, and thus an effective degradation of the chemical by the inoculated microbial strain can take place (Siripattanakul et al., 2009). But as soon as contaminated soils still containing their native microbial population are inoculated, the survival of the added microbes becomes a critical factor. The survival rate of microbes inoculated as free cells into soils mainly depends on a certain selection pressure caused by high (>1000 mg kg^{-1} soil) or middle (50–1000 mg kg^{-1} soil) contaminant concentrations. Under such conditions even horizontal gene transfer from inoculated donor microbes to native soil receptor cells can be observed (Dejonghe et al., 2000) enabling some native microbes to effectively degrade a certain contaminant. Thus, a successful and sustainable inoculation of single strains is feasible in high or middle contaminated soils (Gomes et al., 2005; Lima et al., 2009).

But when considering the sustainability of such inoculation approaches in soils with low contaminant concentrations, like in agricultural soils, an important question arises: is it possible to achieve a long term establishment of an isolated strain after an initially successful phase as soon as the contaminant concentration is considerably reduced? As it can be seen e.g. from the results of Lima et al. (2009) the number of inoculated cells can decrease with time and thus long-term survival of those cells in soils might be questionable. Reasons for such cell reduction include the competition between native and inoculated cells for limited nutrients, along with antagonistic interactions and predation by protozoa and bacteriophages (Gentry et al., 2004). But how to withstand such a trend, and how in general to proceed if even low contamination levels should be avoided e.g. in the case of pesticide residues in agricultural soils? In our previous work (Schroll et al., 2004; Grundmann et al., 2007; Wang et al., 2010), we tested several inoculation approaches and we could show that the use of microbial communities extracted from soils effective in contaminant degradation which were artificially attached to a soil-like carrier material was the most efficient and sustainable approach (Wang et al., 2010). Thus, the question arises: What are the reasons for such very efficient pollutant degradation even at relatively low concentrations of the contaminant in soils?

In soils, bacteria rarely exist as single, isolated entities, but rather as communities comprised of many other species (Atkinson and Williams, 2009; Lowery et al., 2009). To survive in competitive environments, micro-organisms have developed elaborate tactics such as the formation of biofilms (Lowery et al., 2009). In such biofilms microbes have several advantages that are not enjoyed by discrete bacteria; e.g. organisms within biofilms can withstand shear forces, nutrient deprivation, pH changes and antibiotics in a more pronounced manner (Bhinu, 2005; Singh et al., 2006). Biofilms generally consist of cell aggregates, in which a cell-to-cell communication can take place via the exchange of specific signaling molecules (Parsek and Greenberg, 2005). In gram-negative bacteria such signal molecules are formed by N-acyl-homoserine lactone (AHL) structures (Shiner et al., 2005; Camilli and Bassler, 2006; Uroz et al., 2009; Teplitski et al., 2011). The 1,2,4-Trichlorobenzene (1,2,4-TCB) degrading microbial community isolated in our previous study (Wang et al., 2010), consists of gram-negative bacteria; therefore, in the present study we focus on the detection of AHLs.

It was our strategy to identify these signaling molecules in relation to the mineralization of the contaminant 1,2,4-TCB. Positive findings and correlations could be considered as a hint for (i) the existence of a biofilm and (ii) for the higher effectiveness of a biofilm in mineralizing contaminants. We could not conduct this study in soil because there exist a high number of bacteria which produce a large amount of signal molecules, and those would cover the AHLs produced by the inoculated microbial community. Therefore, we performed this study in a more simplified system – liquid cultures – knowing that the out-coming results must be carefully interpreted. It was our main goal to compare the mineralization of 1,2,4-TCB with the formation of AHLs in liquid cultures inoculated (i) with a 1,2,4-TCB degrading microbial community attached to a carrier material (MCCP) and (ii) with the same microbial community without any carrier material (MCLM). To underline and strengthen our conclusions, some results of our former publication (Wang et al., 2010) were included as well.

2. Materials and methods

2.1. Bacteria, medium and chemicals

A 1,2,4-TCB-mineralizing bacterial community containing *Bordetella* sp. as the only 1,2,4-TCB-degrader (Wang et al., 2010) was used. Mineral medium contained 3 g $Na_2HPO_4{\cdot}2H_2O$, 3.5 g KH_2PO_4, 1 g NH_4NO_3, 100 mg $MgSO_4{\cdot}7H_2O$, 50 mg $Ca(NO_3)_2{\cdot}4H_2O$ per liter of ultrapure water (Millipore) with pH 6.5.

Uniformly ^{14}C-ring-labeled 1,2,4-TCB obtained from International Isotope (Munich, Germany) was mixed with non-labeled 1,2,4-TCB (Dr. Ehrenstorfer, Augsburg, Germany) for all experiments resulting in a specific radioactivity of 3.79 Bq μg^{-1}. Scintillation cocktails were obtained from Packard (Dreieich, Germany).

For immunoassay analysis of AHLs, 3-oxo-C_{10}-HSL, Protein G from *Streptococcus*, hydrogen peroxide (H_2O_2), 3,3′,5,5′-tetramethylbenzidine (TMB), and Dimethylsulfoxide (DMSO, 99%), were purchased from Sigma–Aldrich (Steinheim, Germany). All other chemicals and solvents were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Establishing the microbial community on clay particles

To establish the microbial community on clay particles, a liquid culture experiment with 18 sets was performed in 50 mL mineral medium spiked with 1 g of sterile expanded clay particles and with 1 mL liquid medium containing the 1,2,4-TCB mineralizing bacterial community with $(1.4 \pm 0.1) \times 10^7$ *Bordetella* sp. cells (Wang et al., 2010). An amount of 750 μg ^{14}C-1,2,4-TCB dissolved in 25 μL acetone was applied to serve as the carbon source. The liquid culture was incubated in the dark at 100 rpm at 20 ± 1 °C. During the incubation the cultures were aerated twice per week for 1 h at an air exchange rate of 1 L h^{-1}. In order to keep the TCB concentration in the liquid culture nearly constant, 25 μL ^{14}C-1,2,4-TCB (750 μg) were reapplied after each aeration. After 2 weeks, the clay particles were separated from the liquid culture and washed twice with 20 mL PBS (Bertaux et al., 2007) before inoculation.

2.3. Biodegradation experiments

In general, these experiments were conducted (i) to quantify the mineralization of 1,2,4-TCB, (ii) to quantify AHL production during the mineralization process; and finally (iii) to determine the pattern of the AHLs for a selected sampling point at day 4. The comparative experiments were conducted in liquid cultures with 50 mL mineral medium and 25 μL acetonic ^{14}C-1,2,4-TCB (750 μg)-solution inoculated with (i) 1 g clay particles with attached microbial community (MCCP) and (ii) 1 mL liquid medium containing the microbial community (MCLM). Incubation, aeration and reapplication of the liquid cultures were the same as described in Section 2.2. The evolved $^{14}CO_2$ and volatile ^{14}C-substances were trapped separately, followed by a measurement of radioactivity via scintillation counting (Wang et al., 2010). Each set started with 18 replicates. On days 1, 4, 7, 14, 21 and 28 three replicates of each treatment were sacrificed for sample analysis: cell counting via